

CLAIMS

1. A type I restriction-modification enzyme capable of binding to a nucleic acid sequence and also capable of translocating the nucleic acid sequence without causing cleavage thereof, said enzyme having HsdR, HsdM and HsdS sub-units, wherein the HsdR sub-unit is of the type IC restriction-modification enzyme *EcoprrI* or a mutant of said HsdR sub-unit which imparts to the enzyme the property of translocating a nucleic acid sequence without causing cleavage thereof, and without loss of ATPase activity.
2. A molecular motor system comprising a nucleic acid sequence having bound thereto:
- (1) at a first, proximal, region of the nucleic acid, an enzyme capable of translocating the nucleic acid sequence without causing cleavage of the nucleic acid during the translocation, said enzyme remaining bound to the nucleic acid, as a complex therewith, at said proximal region, during the translocation; and
- (2) at a second, distal, region of the nucleic acid, a bound substance capable of remaining bound to the nucleic acid sequence during the translocation, whereby the bound substance becomes translocated, relative to the region of binding of the enzyme to the nucleic acid sequence, as a result of the translocation of the nucleic acid to which it is bound.
3. A system according to Claim 2, wherein the nucleic acid sequence comprises a circular or linear DNA sequence.
4. A system according to Claim 2, wherein the enzyme comprises a type I restriction-modification enzyme having HsdR, HsdS and HsdM sub-units.
5. A system according to Claim 4, wherein the enzyme comprises a type IC restriction-modification enzyme and exhibits the stoichiometric form  $\text{HsdR}_1\text{M}_2\text{S}_1$ .
6. A system according to Claim 4, wherein the HsdR sub-unit is that of the type IC restriction-modification enzyme *EcoprrI* or a mutant of said HsdR sub-unit which

imparts to the enzyme the property of translocating a nucleic acid sequence without causing cleavage thereof, and without loss of ATPase activity.

7. A system according to Claim 2, wherein the bound substance comprises a binding ligand that can bind to a substance in solution.

5 8. A system according to Claim 2, wherein the nucleic acid is attached to a solid support.

9. A system according to Claim 7, wherein the nucleic acid is linear, thus having two ends, the bound substance being bound at one end and a solid support being bound at the other end.

10 10. A system according to Claim 7, wherein the bound substance comprises one or more of:

- (a) a substance which is required to be translocated; or
- (b) means for binding to the nucleic acid-enzyme complex the substance which is required to be translocated; or
- 15 (c) both (a) and (b) together.

11. A system according to Claim 7, wherein the bound substance comprises one or more of:

- (a) a binding ligand for binding a substance in solution, suspension or dispersion;
- 20 (b) an enzyme which produces chemiluminescence;
- (c) a magnetic substance;
- (d) a DNA sequence;
- (e) a scintillant;
- (f) a radioactive substance;
- 25 (g) a substance capable of producing an electric current;
- (h) a substance capable of movement or resulting in movement;
- (i) a substance capable of interacting with the environment of the system to produce a detectable and/or measurable effect; and/or
- (j) biotin, streptavidin or avidin.

12. A molecular motor system comprising a nucleic acid sequence having bound thereto:

(1) an enzyme capable of translocating the nucleic acid sequence without causing cleavage of the nucleic acid during the translocation, said enzyme remaining bound to the nucleic acid, as a complex therewith, at said proximal region, during the translocation; and

(2) a solid support.

13. A molecular motor system according to Claim 12, wherein the enzyme comprises a type I restriction-modification enzyme having HsdR, HsdS and HsdM sub-units.

14. A molecular motor system according to Claim 13, wherein the enzyme comprises a type IC restriction-modification enzyme and exhibits the stoichiometric form HsdR<sub>1</sub>M<sub>2</sub>S<sub>1</sub>.

15. A molecular motor system according to Claim 13, wherein the HsdR sub-unit is that of the type IC restriction-modification enzyme *EcoprrI* or a mutant of said HsdR sub-unit which imparts to the enzyme the property of translocating a nucleic acid sequence without causing cleavage thereof, and without loss of ATPase activity.

16. A method for translocating a substance bound to a nucleic acid sequence from a distal region of the nucleic acid sequence towards a proximal region, which method comprises:

(a) (i) providing at the distal region of the nucleic acid sequence a bound substance, or

(ii) binding a substance to the distal region of the nucleic acid sequence; and

(b) (i) providing at the proximal region a complex of the nucleic acid sequence with an enzyme, or

(ii) complexing an enzyme to the proximal region of the nucleic acid sequence, which enzyme is capable of translocating the nucleic acid sequence without causing cleavage thereof; and

(c) activating the enzyme, whereby the enzyme translocates the nucleic acid sequence, including the bound substance, from the distal region towards the proximal region.

17. A method according to Claim 16, wherein step (c) is carried out in the presence of ATP and  $Mg^{++}$  ions.
18. A method according to Claim 16, wherein step (c) is carried out in the presence of a restriction buffer.
19. A method according to Claim 16, wherein step (c) is carried out in the presence of dithiothreitol.
20. A method according to Claim 16, wherein the polynucleotide-enzyme complex is attached to a solid support.
21. A method according to Claim 16, wherein the nucleic acid sequence comprises a circular or linear DNA sequence.
22. A method according to Claim 16, wherein the enzyme comprises a type I restriction-modification enzyme having HsdR, HsdS and HsdM sub-units.
23. A method according to Claim 21, wherein the enzyme comprises a type IC restriction-modification enzyme and exhibits the stoichiometric form  $HsdR_1M_2S_1$ .
24. A method according to Claim 21, wherein the HsdR sub-unit is that of the type IC restriction-modification enzyme *EcoprrI* or a mutant of said HsdR sub-unit which imparts to the enzyme the property of translocating a nucleic acid sequence without causing cleavage thereof, and without loss of ATPase activity.
25. A method for capturing a test substance in solution, suspension or dispersion and bringing it into association with a solid surface, which method comprises:
- (a) providing a molecular motor system comprising a nucleic acid having:
- (i) a proximal region of the nucleic acid to which is bound an enzyme capable of translocating the nucleic acid sequence without causing cleavage of the nucleic acid during the translocation, said enzyme remaining bound to the nucleic acid, as a complex therewith, at said proximal region, during the translocation;

- (ii) a distal region of the nucleic acid which enables the test substance to be captured, the distal region and/or the test substance being adapted for that purpose; and
- (iii) a solid surface attached to the nucleic acid
- 5 (b) bringing the distal region of the polynucleotide-enzyme complex into contact with the test substance, whereby the test substance is captured; and
- (c) activating the enzyme, whereby the enzyme translocates the polynucleotide, including the test substance, from the distal region towards the solid surface.
- 10 26. A method for screening a test substance for a predetermined biological, chemical or physical activity, which method comprises:
- (a) providing a solution, suspension or dispersion of the test substance, either (i) itself or (ii) in association with a first interactive substance, capable of providing or inducing a detectable reaction in a second interactive substance;
- 15 (b) providing a polynucleotide motor system comprising a nucleic acid having bound thereto:
- (1) at a first, proximal region of the nucleic acid, an enzyme capable of translocating the nucleic acid sequence without causing cleavage of the nucleic acid during the translocation, said enzyme remaining bound to the nucleic acid, as a complex therewith, at said proximal region, during the translocation; and
- 20 (2) at a second, distal region of the nucleic acid, a bound substance capable of remaining bound to the nucleic acid sequence during the translocation, whereby the bound substance becomes translocated, relative to the region of binding of the enzyme to the nucleic acid sequence, as a result of the translocation of the nucleic acid to which it is bound,
- 25 wherein the nucleic acid is attached to a solid support and wherein

09992028.112604

- (i) the bound substance is further capable of binding to a test substance exhibiting the predetermined activity; and
- (ii) the bound substance is itself or the solid support comprises the second interactive substance;
- 5 (c) activating the polynucleotide motor system to effect translocation; and
- (d) monitoring the presence or absence of the detectable reaction during or after translocation.
27. An enzyme according to Claim 1 bound to a nucleic acid sequence.
- 10 28. An enzyme according to Claim 27 wherein the nucleic acid sequence comprises a circular or linear DNA sequence.

0992028 112601